

PENDING CLAIMS

53. A method for detecting a target nucleic acid, comprising;

- a) combining a reactant, a target nucleic acid, an oligonucleotide, and a probe, wherein said probe comprises a charged adduct, under conditions such that said oligonucleotide binds to said target nucleic acid and at least a portion of said probe binds to said target nucleic acid downstream of said oligonucleotide thereby allowing said reactant to cleave said probe releasing a cleaved product comprising said charged adduct; and
- b) detecting said cleaved product.

54. The method of Claim 53, wherein said detection is performed under conditions such that the presence or absence of at least one nucleotide sequence in said target sequence is determined.

55. The method of Claim 53, further comprising a step before step b) of separating said cleaved product with charged-based separation.

56. The method of Claim 53, further comprising a step before step b) of separating said cleaved product with size based separation.

57. The method of Claim 53, wherein said reactant comprises a cleavage agent.

58. The method of Claim 57, wherein said cleavage agent comprises an exonuclease.

59. The method of Claim 57, wherein said cleavage agent comprises an endonuclease.

60. The method of Claim 59, wherein said exonuclease comprises a FEN enzyme.

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61. The method of Claim 53, wherein said reactant comprises a polymerization agent.

62. The method of Claim 53, wherein said charged adduct comprises a linker.

63. The method of Claim 53, wherein said charged adduct comprises a detectable molecule.

64. The method of Claim 63, wherein said detectable molecule is Cy3, Cy5, a fluorescent dye, ethidium bromide, (1,3-propanediamino)-propidium, (diethylenetriamino)-propidium, thiazole orange, (N-N'-tetramethyl-1,2-ethanediamino)-propyl thiazole orange, (N-N'-tetramethyl-1,3-propanediamino)-propyl thiazole orange, totab, toto, EthD, TOED1, TOED2, or FED.

65. The method of Claim 63, wherein said detectable molecule comprises fluorescein.

66. The method of Claim 53, wherein said charged adduct comprises at least one amino acid.

67. The method of Claim 66, wherein said at least one amino acid is lysine, arginine, aspartate, or glutamate.

68. The method of Claim 53, wherein said charged adduct comprises at least one amino-modified base.

69. The method of Claim 53, wherein said charged adduct is located at the 5' end of said probe.

70. The method of Claim 53, wherein said oligonucleotide comprises an extendable primer.

71. The method of Claim 53, wherein said oligonucleotide comprises an INVADER oligonucleotide.

72. The method of Claim 53, wherein said probe comprises an uncleavable region.

73. The method of Claim 72, wherein said charged adduct is attached to said uncleavable region of said probe.

74. The method of Claim 53, wherein said cleaved product comprises an uncleavable region.

75. The method of Claim 53, wherein said cleaved product comprises one or more phosphonate groups, or one or more phosphorothioate groups.

76. The method of Claim 53, wherein the charge-to-mass ratio of said cleaved product is different from the charge-to-mass ratio of said probe.

77. A method for detecting a target nucleic acid, comprising;

- combining reactants, target nucleic acids, and a pair of detection nucleic acids for each target nucleic acid to be detected, wherein each pair of detection nucleic acids comprises an oligonucleotide, and a probe, and wherein said probe comprises a charged adduct, and wherein said combining is performed under conditions such that each of said pairs of nucleic acids binds one of said target nucleic acids under conditions such that at least a portion of said probe binds to said target nucleic acid downstream of said oligonucleotide thereby allowing said reactant to cleave said probe, thereby releasing cleaved products comprising said charged adduct; and
- detecting said cleaved products.

78. The method of Claim 77, wherein said detection is performed under conditions such that the presence or absence of at least one nucleotide sequence in at least two target sequences is determined.

79. The method of Claim 77, further comprising a step before step b) of separating said cleaved products with charged-based separation.

80. The method of Claim 77, further comprising a step before step b) of separating said cleaved products with size based separation.

81. The method of Claim 77, wherein said reactants comprise a cleavage agent.

82. The method of Claim 81, wherein said cleavage agent comprises an exonuclease.

83. The method of Claim 77, wherein said cleavage agent comprises an endonuclease.

84. The method of Claim 83, wherein said exonuclease comprises a FEN enzyme.

85. The method of Claim 77, wherein said reactants comprise a polymerization agent.

86. The method of Claim 77, wherein said charged adducts comprise a linker.

87. The method of Claim 77, wherein said charged adducts comprise a detectable molecule.

88. The method of Claim 87, wherein said detectable molecule is Cy3, Cy5, a fluorescent dye, ethidium bromide, (1,3-propanediamino)-propidium, (diethylenetriamino)-propidium, thiazole orange, (N-N'-tetramethyl-1,2-ethanediamino)-propyl thiazole orange, (N-

N¹-tetramethyl-1,3-propanediamino)-propyl thiazole orange, totab, toto, EthD, TOED1, TOED2, or FED.

89. The method of Claim 87, wherein said detectable molecule comprises fluorescein.

90. The method of Claim 77, wherein said charged adducts comprise at least one amino acid.

91. The method of Claim 90, wherein said at least one amino acid is lysine, arginine, aspartate, or glutamate.

92. The method of Claim 77, wherein said charged adducts comprise at least one amino-modified base.

93. The method of Claim 77, wherein said charged adducts are located at the 5' end of said probes.

94. The method of Claim 77, wherein each of said oligonucleotides comprises an extendable primer.

95. The method of Claim 77, wherein each of said oligonucleotides comprises an INVADER oligonucleotide.

96. The method of Claim 77, wherein each of said probes comprises an uncleavable region.

97. The method of Claim 96, wherein said charged adducts are attached to said uncleavable region of said probes.

98. The method of Claim 77, wherein said cleaved products comprise an uncleavable region.

99. The method of Claim 77, wherein said cleaved products comprise one or more phosphonate groups, or one or more phosphorothioate groups.

100. The method of Claim 77, wherein the charge-to-mass ratio of said cleaved products are different from the charge-to-mass ratio of said probes.

101. A kit comprising:

- a) a cleavage agent;
- b) a first oligonucleotide comprising: i) a charged adduct, and ii) a portion completely complementary to a first region of a target nucleic acid; and
- c) a second oligonucleotide comprising a 3' portion and a 5' portion, said 5' portion completely complementary to a second region of said target nucleic acid downstream of and contiguous to said first portion.

102. The kit of Claim 101, wherein said 3' portion of said second oligonucleotide comprises a 3' terminal nucleotide not complementary to said target nucleic acid.

103. The kit of Claim 101, wherein said 3' portion of said second oligonucleotide consists of a single nucleotide not complementary to said target nucleic acid.

104. The kit of Claim 101, wherein said kit further comprises a solid support.

105. The kit of Claim 104, wherein said first oligonucleotide is attached to said solid support.

106. The kit of Claim 104, wherein said second oligonucleotide is attached to said solid support.

107. The kit of Claim 101, wherein said cleavage agent comprises a structure-specific nuclease.

108. The kit of Claim 107, wherein said structure-specific nuclease comprises a thermostable structure-specific nuclease.

109. The kit of Claim 101, wherein said cleavage agent comprises a 5' nuclease.

110. The kit of Claim 109, wherein said 5' nuclease comprises a thermostable 5' nuclease.

111. The kit of Claim 109, wherein a portion of the amino acid sequence of said nuclease is homologous to a portion of the amino acid sequence of a thermostable DNA polymerase derived from a thermophilic organism.

112. The kit of Claim 101, further comprising a buffer solution.

113. The kit of Claim 101, further comprising providing a third oligonucleotide complementary to a third portion of said target nucleic acid upstream of said first portion of said first target nucleic acid.

114. The kit of Claim 101, further comprising said target nucleic acid.

115. The kit of Claim 101, further comprising a second target nucleic acid.

116. The method of Claim 101, wherein said charged adduct comprises a linker.

117. The method of Claim 101, wherein said charged adduct comprises a detectable molecule.

118. The method of Claim 117, wherein said detectable molecule is Cy3, Cy5, a fluorescent dye, ethidium bromide, (1,3-propanediamino)-propidium, (diethylenetriamino)-propidium, thiazole orange, (N-N'-tetramethyl-1,2-ethanediamino)-propyl thiazole orange, (N-N'-tetramethyl-1,3-propanediamino)-propyl thiazole orange, totab, toto, EthD, TOED1, TOED2, or FED.

119. The method of Claim 117, wherein said detectable molecule comprises fluorescein.

120. The method of Claim 101, wherein said charged adduct comprises at least one amino acid.

121. The method of Claim 120, wherein said at least one amino acid is lysine, arginine, aspartate, or glutamate.

122. The method of Claim 101, wherein said charged adduct comprises at least one amino-modified base.

123. The method of Claim 101, wherein said charged adduct is located at the 5' end of said first oligonucleotide.

124. The method of Claim 101, wherein said first oligonucleotide comprises an uncleavable region.

125. The method of Claim 124, wherein said charged adduct is attached to said uncleavable region of said first oligonucleotide.